Discovery. 1:126, 2004; Exp Hematol. 32:470, 2004; Exp Hematol. 32:300, 2004; Stem Cells. In press). In this first phase I clinical trial of CTCE-0214, the safety, tolerability, pharmacokinetic profile, and the pharmacodynamic effects of single subcutaneous doses of CTCE-0214 given to healthy subjects were studied. The randomized, double-blinded, placebo-controlled dose-escalation trial enrolled 24 subjects in 6 dose-escalation groups. Four healthy human subjects in each cohort received either CTCE-0214 or Placebo, randomized in a 3:1 ratio. The doses of CTCE-0214 were 0.2 mg/kg, 0.5 mg/kg, 0.8 mg/kg, 1.5 mg/kg, 2.0 mg/kg, and 3.0 mg/kg. CTCE-0214 was shown to be safe with no serious adverse events reported. The most common drug related adverse events were injection site pain and erythema, which were transient and resolved without intervention. The severity of injection site pain appeared to be associated with the overall quantity of study drug administered. Moderate or severe pain was reported only at 80 mg or greater of CTCE-0214 per syringe. Dilution across more than one syringe appeared to effectively reduce injection site pain. Pharmacokinetic analysis of CTCE-0214 plasma concentrations showed that T_{max} was reached at 0.25 hours post-administration for all CTCE-0214 treated cohorts. The apparent terminal elimination half-life ($t_{1/2}$) values were estimated to be 0.41 to 0.32 hours. CTCE-0214 administration was associated with significant dose-dependent increases in total white blood cell and neutrophil counts in treated subjects, peaking at around 6 hours post-injection. In the 3.0 mg/kg arm, the mean difference in neutrophil count from baseline was more than three times that of the Placebo arm. The same trend was apparent in the 2.0 and 1.5 mg/kg arms. These results suggest that SDF-1 agonists may potentially be used in patients with low neutrophil count receiving chemotherapy with or without the use of G-CSF. The potential for CTCE-0214 to mobilize neutrophils and other blood cells merits serious consideration.

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DYSKERATOSIS CONGENITA: A HUMAN MODEL TO STUDY THE ROLE OF TELOMERASE AND TELOMERE LENGTH IN HEMATOPOIESIS

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Dyskeratosis congenita (DC) is an inherited multisystem disorder of premature aging characterized by early bone marrow (BM) failure. This disease is due to telomerase defects resulting in telomere shortening and premature senescence of various somatic cells, particularly those from highly proliferative tissues. Telomerase plays an important though not fully understood role in hematopoiesis. We have recently identified a three-generation kindred with autosomal dominant DC with affected individuals having a single gene deletion of the RNA component of telomerase, hTERC, and recent third generation patients are displaying signs of BM dysfunction at an earlier age than their affected parents. To explain these findings, we hypothesize that early BM failure in DC may be due to a proliferative/differentiative defect in hematopoietic stem cells (HSC) as a consequence of telomere shortening. Five patients (age 11–50) from 2 successive generations have thus far been enrolled on a study that allows collection of HSC by leukopheresis. All patients had clinical and laboratory features of DC, including significant BM hypocellularity (10–30%). The percentage of CD34+ cells in the G-CSF primed leukopheresis product was significantly lower in DC subjects (0.05–0.24%) compared to controls (0.7–1.2%). Extensive immunophenotyping of HSC revealed a higher percentage of CD33+/CD34+ cells in DC subjects (60–97%) relative to controls (9–28%). HSC function was examined in vitro by a clonogenic progenitor cell assay, assessing percent, number, and morphology of colony forming units (CFU). Of note, 2/5 DC subjects (both third generation) had a markedly reduced number of CFU, and percentage of CFU-GM and CFU-GEMM were decreased in 4/5 DC subjects relative to controls. To assess DC HSC function in vivo, CD34+ HSC from 2 different DC subjects and age-matched controls were injected into sublethally irradiated NOD-SCID mice. None of the mice injected with DC HSC showed evidence of human hematopoiesis at up to 20 weeks post injection, while animals receiving control HSC had evidence of human hematopoiesis by 6 weeks. In conclusion, DC is an excellent human model to assess the role of telomerase and telomere shortening in hematopoiesis. Importantly, there appears to be a correlation of the DC clinical phenotype and DC HSC function with telomere length. Further studies are needed to determine whether the aberrant function of DC HSC can be rescued by a gene-transfer approach.

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GLYCOGEN SYNTHASE KINASE-3 IS AN IN VIVO REGULATOR OF HUMAN HEMOPOIETIC STEM CELLS

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Hemopoietic stem cells (HSCs) are rare cells of the hemopoietic system with the ability to self-renew and differentiate into mature blood cells. The application of HSC transplantation as a curative intervention can be limited by the lack of a suitable donor or the suboptimal collection of HSCs. Umbilical cord blood (UCB) cells have potential as a readily available alternate source of HSCs but at present have limited utility in adult recipients because the relatively low numbers of HSCs can lead to delayed hemopoietic reconstitution. At present, there exists no therapeutic intervention that allows for the in vivo expansion of transplanted HSCs. We examined the effects of inhibiting glycogen synthase kinase-3 (GSK-3), a constitutively active serine/threonine kinase, on the regulation of the human HSC pool. Primitive lineage-depleted (Lin-) neonatal UCB cells or adult G-CSF mobilized peripheral blood (M-PB) cells were intravenously injected into sublethally irradiated NOD/SCID mice. The mice were then treated with either 30 mg/kg of a GSK-3 inhibitor or with vehicle twice per week for the duration of the 5 to 6 week transplant period. NOD/SCID recipients treated with the GSK-3 inhibitor demonstrated an increased frequency and total number of primitive human HSCs (CD45+ CD34+) after transplantation of UCB-derived HSCs compared to vehicle-treated controls. The mean number of HSCs in the bone marrow were 12791 ± 2892 versus 62374 ± 15533, respectively, (mean ± SEM; $P = .02$; N = 4 mice/group). To further characterize the effect of GSK-3 inhibition on human HSC repopulating capacity, sorted CD45+ human hematopoietic cells from the reconstituted mice were plated in a functional clonogenic in vitro assay. Hemopoietic cells derived from recipients reconstituted with UCB or M-PB-HSCs and treated with GSK-3 inhibitor generated greater numbers of HSC-colony forming units (HSC-CFU) than untreated controls (mean ± SEM: 2121 ± 526 versus 1021 ± 356; respectively. $P = .038$, N = 7). The developmental potential of the HSC-CFU to form mature colonies of various subtypes (erythroid, granulocyte, macrophage, and granulocyte-macrophage) was not altered by the treatment. Our results demonstrate that GSK-3 can modulate HSC activity in vivo and suggest that administration of GSK-3 inhibitor may provide a clinical means to directly enhance the repopulating capacity of transplanted HSCs.

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MULTIPOTENT ADULT PROGENITOR CELLS (MAPC) ARE NON-IMMUNOGENIC AND DISPLAY IMMUNOSUPPRESSIVE PROPERTIES ON ACTIVATED T CELLS

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Multipotent Adult Progenitor cells (MAPC) are nonhematopoietic stem cells, present within the bone marrow that can be grown extensively and can be induced to generate cells with phenotypic characteristics and gene profile of cells derived from endoderm, ectoderm and mesoderm. The capacity to differentiate into these

diverse cell types distinguishes MAPC from Mesenchymal Stem Cells (MSC), and suggests that MAPCs may therefore be an ideal cell for in vivo therapies for tissue repair or regeneration in multiple organ systems (Jiang Y et al Nature, 2002; 418:41–49). We have developed the technology for the large-scale expansion of MAPC with stable phenotype and biological properties. Under these conditions, cell surface marker analysis of MAPC revealed that these cells are positive for CD10, CD90, and CD49c, they are weakly positive for MHC class I, and are negative for MHC class II, CD45, and CD106, indicating that MAPC are not derived from the hematopoietic lineage. However, to optimize in vivo utilization, the understanding of immunological properties of MAPC is critical. Thus, we undertook an analysis of MAPC immunogenicity. First, these stem cells were shown to be non-immunogenic as MAPC from two different rat strains did not stimulate allogeneic T cell proliferation, while splenocytes of the same rat strains elicited strong proliferative responses in a mixed lymphocytic culture. Second, MAPC displayed immunosuppressive properties. Addition of MAPC at the initiation of a mixed lymphocyte reaction (MLR) suppressed T cell proliferation in a dose dependent manner. The inhibition was detectable when number as low as 3000 MAPC/well were added to 1 × 105 T cell responder. Lymphocyte proliferation in MAPC-containing cultures was inhibited by up to 80% when compared to cultures without MAPC. The ability to inhibit T cell alloresponse was independent of the MHC, allowing the use of third party MAPC as inhibitory cells. MAPC also inhibited proliferative responses to the T cell mitogen Concanavalin A (50%), although inhibition required higher MAPC:responder cell ratios. Taken together, these data suggest that MAPC are progenitor cells that do not express markers of the hematopoietic lineage; they are non-immunogenic to T cells, suggesting that universal MAPC donors may be used for tissue repair or regeneration; and MAPC exhibit potent immunosuppressive properties, a result which suggests that these calls may be useful in the management and/or prevention of GVHD.

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CYTOKINE-INDUCED IN VIVO EXPANSION AND MOBILIZATION OF MARROW MESENCHYMAL STEM CELLS IN NONHUMAN PRIMATES

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Mesenchymal stem cells (MSC), as well as showing promise for tissue regeneration and gene therapies, have the potential to facilitate bone marrow transplantation. First, they can provide stromal support for engrafted cells, and second, their immunologic properties may facilitate engraftment and reduce graft versus host disease. Much is made of the ease with which MSC can be cultured and expanded many-fold in vitro, however there is evidence that this can alter the fundamental properties of these cells. Therefore, we have explored the potential to increase the number of marrow MSC in vivo using various cytokine regimens in a nonhuman primate (NHP) model. Male baboons between 7 and 11 years of age received subcutaneous cytokines as follows: (1) G-CSF 100 mcg/kg/day for 5 days; (2) pegylated G-CSF (pegG-CSF), single dose 300 mcg/kg day −5; (3) G-CSF 100 mcg/kg/day + stem cell factor (SCF) 50 mcg/kg/day for 5 days; and (4) pegylated megakaryocyte growth and development factor (pegMGDF) 1 mcg/kg second daily for 10 days + G-CSF 100 mcg/kg/day for 5 days starting day −5 before the harvest. Bone marrow was aspirated from the iliac crest at baseline and on the final day of cytokine administration. Mononuclear cells were isolated on Ficoll-Paque Plus, and plated in triplicate in alpha-MEM plus 20% FCS for fibroblast colony forming cells (CFU-F). Cells were fixed, stained, and scored on day 7, and the number of CFU-F/mL of bone marrow was then calculated. The mean baseline CFU-F marrow concentration was 2 032/mL (n = 15). There was an increase in CFU-Fs following each of the cytokine regimens to 11 427/mL, 8 430/mL, 20 662/mL, and 15 745/mL after G-CSF (n = 3, $P < .001$), pegG-CSF (n = 4, $P > .05$), G-CSF+SCF (n = 5, $P < .001$), and G-CSF+pegMGDF (n = 4, $P < .01$) compared to baseline, respectively. We also explored the potential for the mobilization of MSC into peripheral blood. CFU-F were not detected in baseline peripheral blood mononuclear cells (PBMNC) samples from any animal. However, CFU-F were detected in 3 animals after G-CSF+SCF at a frequency of 0.8/mL to 1.5/mL, but no other cytokine regimen. In conclusion, these data confirm that cytokine regimens used to mobilize hemopoietic stem cells can be used to induce in vivo expansion and mobilization of MSC and that the combination of G-CSF and SCF may be the most effective.

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MODULATION OF ALDEHYDE DEHYDROGENASE ACTIVITIY AND RETINOID SIGNALING INDUCES HEMATOPOIETIC STEM CELL SELF RENEWAL

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Aldehyde dehydrogenases are cytosolic enzymes that convert aldehydes into carboxylic acids. Human aldehyde dehydrogenase 1 (ALDH1) is highly expressed in the liver and in hematopoietic stem cells (HSCs). Although ALDH1 is a selectable marker of HSCs, its HSC-specific function is unknown. We hypothesized that ALDH might play a critical role in HSC fate determinations since it is required for the production of retinoic acids, which are broadly implicated in tissue differentiation, tissue patterning, and embryonic development in vertebrates. In this study, highly purified human CD34+CD38−lin− HSCs were cultivated with early acting cytokines, thrombopoietin, stem cell factor, and Flt-3 ligand (TSF) in the presence or absence of the competitive ALDH inhibitor, diethylaminobenzaldehyde (DEAB). Treatment of human BM and CB HSCs with TSF caused a loss of CD34+CD38− cells in culture, morphologic differentiation, amplification of committed colony forming cells (CFCs), and a loss of primitive cells capable of repopulating non-obese diabetic/severe combined immunedeficient mice (SCID-Repopulating Cells, SRCs). Conversely, culture of BM and CB HSCs with TSF plus 100 μM DEAB blocked the morphologic differentiation and lineage commitment of HSCs in culture, expanded the CD34+CD38− population, and amplified SRCs 2- to 4-fold compared to input, indicating a fundamental role for ALDH activity in HSC differentiation. The effects of DEAB could be reversed by the co-administration of the retinoic acid receptor (RAR) agonist, all-trans retinoic acid (ATRA), suggesting that the ability of ALDH to produce retinoic acids is important in determining HSC fate. Via screening studies of direct ligands of RAR and RXR, we also identified a selective RXR modulator, LGD101506, which functioned similarly to DEAB in impeding HSC differentiation and causing the 4-fold expansion of SRCs. Interestingly, both DEAB treatment and LGD101506 reversed the down-modulation of HOXB4 transcription that was otherwise observed in CD34+CD38−lin− cells during culture with cytokines, suggesting that inhibition of ALDH or RXR modulation may promote HSC self-renewal via discrete interactions with other regulatory pathways. Modulation of ALDH activity and retinoid signaling is a novel and effective strategy to amplify human HSCs.

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DEXAMETHASONE ENHANCES ERYTHROPOIETIC DIFFERENTIATION OF EMBRYONIC STEM CELLS

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We have studied the in vitro differentiation of murine embryonic stem cells (ES) towards erythropoiesis and expression of genes during this process. It has been reported that dexamethasone directs ES cells towards erythrocytic differentiation but the mechanism of gene regulation induced by dexamethasone is not well understood. We hypothesized that dexamethasone induces upregulation of erythropoietic genes such as GATA-1, FLK-1, EPO-R, and directs ES cells towards erythropoietic differentiation.